Distributional dynamics of a specialized subterranean community oppose the classical understanding of the preferred subterranean habitats

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Abstract. The most specialized organisms to living in the subterranean environment, i.e., troglobionts, reside prevalently in the deepest cave zone and shallow subterranean habitats and complete their whole life cycles therein. Since troglobionts in most caves depend on surface organic matter resources, we hypothesized that they could also select the surface-most cave sections, so far the other environmental conditions there are

favorable. Over one year, we analyzed the annual distributional dynamics of a subterranean community consisting of 17 troglobionts species, in monthly intervals in relation with multiple environmental parameters. Cumulative standardized annual species richness and diversity clearly indicated the existence of two ecotones within the cave: between soil and shallow subterranean habitats, inhabited by soil and shallow troglobionts, and between the transition and inner cave zones, where shallow and deep troglobionts overlap their spatial niches. The mean standardized annual species richness and diversity showed inverse courses, but both contributed to a better insight in the dynamics of subterranean fauna. Regression analyses revealed that temperatures between 7–10°C, high ground substrate moisture, large cross-sections size and high ground substrate pH were the most important ecological drivers governing the spatio-temporal dynamics of troglobionts. Overall, this study show general trends in the annual distributional dynamics of troglobionts in shallow caves and reveal that the distribution patterns of troglobionts within subterranean habitats may be more complex than commonly assumed.

Additional key words: cave community, ecological classification, ecotone, pitfall trapping, seasonality, spatio-temporal dynamics

Beneath the Earth's surface, various kinds of natural and artificial cavities occur, from large cave chambers, to millimeter-sized interspaces between sand grains (Ford & Williams 2007; Palmer 2007; Culver & Pipan 2009a, b, 2014). Subterranean habitats share some common environmental features, which differ distinctively from those found on the surface. Subterranean environment is generally characterized by perpetual

darkness and reduced variability of abiotic parameters (Culver & Pipan 2009b; Hüppop 2012). However, seasonal variation in temperature, relative air humidity and some other parameters still occurs, which reflect a delayed response to changing weather on the surface (Tobin et al. 2013). Surface climate mostly affects the cave sections closest to the entrance, while deeper inside the cave, environmental conditions are much more stable and the surface effects temporally lag behind (Cigna 2002; Badino 2010; Tobin et al. 2013; Šebela & Turk 2014; Mammola et al. 2015). Another essential difference compared to the surface environment is the absence of primary producers in most subterranean habitats (Simon et al. 2007; Engel 2012). Consequently, the subterranean fauna mostly depends on organic matter input from surface habitats (Gers 1998; Poulson & Lavoie 2000), and is generally poorly diversified (Gibert & Deharveng 2002). In spite of this, numerous investigations have disclosed a much higher biodiversity and a higher degree of endemism among subterranean species than predicted previously (e.g., Peck 1998; Culver & Sket 2000; Elliott 2000; Christman et al. 2005; Pipan & Culver 2007a, b; Zagmajster et al. 2008; Reboleira et al. 2011; Deharveng et al. 2012).

This diversified subterranean fauna includes temporary and obligate residents of the subterranean habitats (Sket 2008; Culver & Pipan 2009a, 2014; Trajano & Carvalho 2017). Schiner (1854) and Racoviţă (1907) grounded the ecological classification of the subterranean fauna. Despite since then many classifications have been proposed (reviewed in Sket 2008; Trajano & Carvalho 2017), this remains one of the most frequently referred classification. Schiner and Racoviţă classified subterranean fauna into three ecological groups: (1) Trogloxenes, which do not complete their life cycle in subterranean habitats. Accordingly, they should be the most abundant in the entrance

cave section. (2) Troglophiles complete their life cycles either in the subterranean or in the surface environments, forming populations in both habitats (Sket 2008; Mammola 2018). Some populations do, while others do not complete their life cycle underground. (3) Specialized taxa, troglobionts, complete the whole life cycle in the subterranean environment (Culver & Pipan 2009a; Romero 2009; Assmann et al. 2010; Novak et al. 2014). These species are referred to occupy the inner, most stable, cave zone.

The spatial distribution of these three ecological groups within subterranean habitats is a consequence of variability in environmental parameters and species response to this variability mostly on account of seasonal changes in the regional and local climate (Novak et al. 2004; Tobin et al. 2013; Lunghi et al. 2017). Classically, it has been observed that there is a gradient of subterranean specialization of the biological community from the entrance zone toward the deepest sectors (Mammola 2018). Accordingly, the community in the vicinity of the surface is usually dominated by trogloxenes and troglophiles, whereas troglobionts became progressively more abundant at greater depths (Schiner 1854; Racoviţă 1907; Poulson & White 1969; Culver & Poulson, 1970; Howarth 1983; Tobin et al. 2013). However, recent studies disclosed that distributions of taxa in subterranean habitats do not always comply with this general pattern (e.g., Novak et al. 2012, Pellegrini & Ferreira 2016). Surface organisms are typically observed even at great depths in caves, for example surface dipterans have been observed at –2140 m in the Krubera-Voronja Cave, Western Caucasus (Sendra & Reboleira 2012). At the same time, by studying 54 cave communities in Slovenia, Novak et al. (2012) showed that troglobionts may consist of two spatially segregated groups of species: the shallow and the deep troglobionts, the first being adapted to the shallow subterranean habitats and the second to deep subterranean habitats. This, in

general, supports the Racoviţă (1907) opinion that many subterranean species normally live in fissures and appear in caves by hazard.

So far, subterranean habitats have been investigated for their environmental conditions in relation to their associated fauna (e.g., Pipan 2005; Culver & Pipan 2010; Tobin et al. 2013; Lunghi et al. 2014, 2017; Prous et al. 2015; Mammola et al. 2015, 2017; Bento et al. 2016). Distributional dynamics of subterranean fauna, especially troglobionts, have rarely been studied. Juberthie (1969) observed daily migrations of the troglobiotic beetle *Aphaenops cerberus* (Coleoptera: Carabidae) between a spacious cave and adjacent fissures, driven by changing air movements within the cave; the beetles promptly reacted to changes in microclimatic conditions and appeared in the cave in a calm only. The same was observed in *Leptodirus hochenwartii* (Coleoptera: Leiodidae) and *Anophthalmus hirtus* (Coleoptera: Carabidae) (Novak et al. 1980). Crouau-Roy et al. (1992) studied the dynamics of different populations of *Speonomus hydrophilus* (Coleoptera: Leiodidae) in a cave and in adjacent network of superficial interspaces in-between clastic substrate−the so-called *milieu souterrain superficiel* (MSS)−and observed conspicuous fluctuation in population size and vertical migration over the year. This was triggered by seasonal variation of environmental parameters, especially temperature and relative air humidity. Significant vertical migration between MSS and caves owing to seasonal fluctuations in temperature has also been recorded in other subterranean species (Chapman 1985; Polak 2012; Mammola et al. 2016). Beside variation in climatic conditions, trophic availability was found to be an important factor affecting the spatial distribution of several spider species inhabiting caves (e.g., Novak et al. 2010; Manenti et al. 2015; Mammola & Isaia 2014, 2016; Lunghi 2018). Furthermore, the spatial distribution of particular subterranean species is also affected

by the structural complexity of cave walls (e.g., Bourne 1976, 1977; Mammola & Isaia 2014, 2016).

While evident patterns in distributions of single troglobiotic species in caves are reported, great gaps remains in knowledge about distributional dynamics of the whole troglobiotic community. Despite extensive research in recent decades, subterranean organisms remain among the least understood groups in the world (Soares & Niemiller 2013). Under this perspective, the study of distributional dynamics of troglobionts may help us to achieve a better understanding of their preferred subterranean habitats and microhabitats. In the present research, we studied the annual dynamics of troglobiont community in a cave in Slovenia, with the aim of exploring whether troglobionts could occur in the surface-most cave sections, rather than exclusively in the deepest cave sectors, and to find out which environmental parameters would influence such distribution. We hypothesized that: (1) Variation in the environmental conditions along the cave length primarily drive spatial dynamics of troglobionts. (2) Seasonal variation in the environmental parameters determine parallel seasonal changes in troglobiotic community along the cave, eventually permitting the colonization of surface-most sector during favorable periods.

Methods

Study site

We carried out the study in the cave Zguba jama (Cadastre number 6290; E 14°12’49.57”, N 45°47’47.50”; 561 m a.s.l.; Cave Register Karst Research Institute

ZRC SAZU and Speleological Association of Slovenia) near Postojna, Slovenia (Fig. 1). This relatively small, horizontal cave is 122 m long, with a 1.5 m wide by 1 m high entrance. This study site was chosen owing to its simple morphology, linear shape and nearly constant cross-section (2 x 2 m average cross-section, with a few largest sites where the ceiling reaches a 5-m-height), allowing us to sample the species and measuring the considered environmental parameters in a more standardized way. The cave is in close vicinity of the Postojna cave (Šebela 1994), one of the world's hotspots of subterranean fauna (Culver & Sket 2000; Zagmajster et al. 2008; Sket 2012; Culver & Pipan 2013). It hosts a rich and diverse community, consisting of more than 100 species of which at least 17 can be classified as troglobionts (Kozel et al. 2017).

Sampling design

To study the distributional dynamics of troglobionts, we defined 31, on average 4 m long, sampling sections in the cave (Fig. 2). The cave was surveyed twice a month within about 48 hours over one year, from March 2012 to February 2013. The environmental parameters, which may vary considerably in subterranean microhabitats across one year (Cigna 2002, Badino 2010), were measured monthly at the first monthly survey in each sampling site. We took monthly records of the air temperature (Tair) and relative air humidity (RH) at the ground and below the ceiling using a handheld aspiration psychrometer (Ahorn FN A846, Germany) fastened on a 2 m long stick to diminish disturbance by the researcher. A sonde thermometer (Checktemp 1, Hanna, Germany) was used to measure the ground temperature (Tgr) at a depth of 2 cm. The substrate samples (ca. 100 g) were collected at ca. 0.25 m^2 area around the pitfall traps.

169The substrate moisture content (MC) was determined after drying samples at 105°C in 170the laboratory heating oven until reaching the stable weight. Conversely, more stable 171parameters over the year were measured only once, during the first survey. The pH of 172the ground substrate (pHgr) was measured with a pH-meter (Hanna Instruments HI221 173Calibration Check, Microprocessor pH Meter, Woonsocket, RI, USA), and the carbonate 174content (CC) in the substrate by means of Scheibler's calcimetry. Organic material 175content (OM) was determined after the ignition of dry substrate samples at 650°C in a 176muffle furnace until reaching a constant weight. The distance from the entrance (DisE; 177polygonal distance) and from the surface (DisS; vertical distance) of the center of the 178sampling sections were calculated upon the ground cave plan and terrain profile above 179the cave. The passage cross-section size (Size) and the sampling section area (Area), 180i.e., wall and ceiling surface area, were, simplified, considered as being an open 181cylinder. The average mean height and width of each sampling section were used to 182calculate the radius of the circle. Chimneys and concavities were additionally taken into 183account.

184 The fauna was recorded by visual inspection and by pitfall trapping. We applied 185simultaneously two sampling methods, since it is documented that complementary 186sampling approaches maximize the detection and completeness of diversity of cave-187dwelling arthropods (Kozel et al. 2017; Wynne et al. 2018, 2019).

188 For visual inspection, we devoted 0.5 minute/m^2/observer. Only individuals that we 189could not identify to the species level in the cave were taken in the laboratory for 190identification. In order to investigate simultaneously the fauna occurring on the ground 191and on walls (Kozel et al. 2017), we used two parallel sets of pitfall traps: the ground set 192and the upper set−close below the ceiling−along the cave (Fig. 2). Pitfall traps were

baited with decomposing beef meat and apple juice with a lacing of cherry and maraschino essence, and a few drops of detergent to reduce the surface tension. Sampling of fauna was carried out within two visits a month. At the first visit, we visually inspected the cave for the fauna and set the traps. At the second visit, within about 48 hours, we picked up the traps and took them into laboratory for identification. The voucher specimens preserved in 70% ethanol are deposited at the Faculty of Natural Sciences and Mathematics, University of Maribor. For details on the traps see Kozel et al. (2017). Counts deriving from visual inspection and pitfall trapping were merged in the analysis (Wynne et al. 2019).

Data analysis

To graphically visualize the overall environmental conditions along the cave, microclimatic and physical parameters were mapped using ArcGIS Desktop 10.3.1 (Esri 2015). We created a vector layer of the cave, projecting it “on the fly” over the original raster scheme of the cave. The point station data were interpolated by Inverse Distance Weighted (IDW) tool, using a power of 2 as exponent of distance and a cell size of 2 for all resulting grid maps.

The sampling sections on each sampling date were used as basic sample units. Mean values of Tair and RH from the ground and below-ceiling measurements, and the distances from the entrance and from the surface were used in the statistical analyses. Data on fauna recorded by visual inspection and pitfall trapping were pooled for the analyses.

In order to investigate the annual dynamics of troglobionts with respect to the environmental parameters, we applied generalized linear mixed models (GLMMs). The mixed procedure allowed us to deal with replicated prospections of the sampling section, by specifying the sampling section as a random factor. We modeled abundance and presence-absence data of troglobionts separately. In the abundance approach, we used counts of individuals deriving from pitfall and visual inspections as the response variable. To adjust the abundance for the different sizes of the sampling sections, we included the sampling section area (Area) as an offset in the model.

Given the low richness of species per sampling section (ranging from 0 to 4 species, with most values being either 0 or 1), we expressed richness as presence or absence of troglobionts within the sampling plot (i.e., Bernoulli distribution 0–1). This model allowed us to uncover fundamental conditions governing the presence of troglobionts in the cave, thus complementing the abundance approach.

The following independent variables were addressed as potential predictors of the dependent variables: distance from the entrance (DisE), distance from the surface (DisS), Season (Sea; consisting of four levels: “Spring”, “Summer”, “Autumn”, “Winter”), ground temperature (Tgr), air temperature (Tair), relative air humidity (RH), substrate moisture content (MC), ground substrate pH (pHgr), organic material content (OM), carbonate content (CC), and passage cross-section size (Size). All variables but season were continuous.

Prior to model fitting, a systematic data exploration was carried out following the protocol proposed by Zuur et al. (2010). Including outliers or highly correlated variables increase type I and II statistical errors and may potentially result in incorrect ecological conclusions (Zuur et al. 2009, 2010). Accordingly, Cleveland dotplots were used to

assess the presence of outliers in response and explanatory variables and multi-collinearity among continuous variables was tested using Pearson correlation coefficients and variance inflation factors (VIF), setting $r \geq \pm 0.7$ and VIF > 3 as cut-off values (Zuur et al. 2013). Collinearity between continuous variables and the categorical variable Season was assessed graphically through boxplots. Possible non-linear effects of the continuous variables included in the models were investigated with generalised additive models (GAM), using the *gam* R package (Hastie 2016). Coplots were further constructed to check for potential interactions between Season and the continuous covariates.

The abundance of troglobionts was modeled choosing a negative binomial distribution family, the distribution applied for over-dispersed count data (Zuur et al. 2009). The presence-absence of troglobionts was modeled through Binomial GLMM using a complementary log-log link (clog-log), owing to unbalanced zeros (absences) and ones (presences) in the dataset (Zuur et al. 2009). All models were fitted through the glmer command from the *lme4* R package (Bates et al. 2016).

After fitting the initial models, including all non-collinear covariates of interest, we conducted model selection (Johnson & Omland 2004). A backward elimination procedure was chosen, whereby variables were progressively deleted according to AICc values (Burnham & Anderson, 2002). AICc values were calculated in the *MuMIn* R package (Bartón 2018). The backward elimination procedure was reiterated manually until a minimum adequate model consisting exclusively of statistically significant variables was obtained. Model validation was carried out on the final models in accordance with Zuur et al. (2009, 2013). Factor significance was checked via likelihood ratio test.

Species richness and diversity of troglobionts for each sampling section and each month were calculated. Species diversity was calculated through the Shannon-Weaver index (H') for each sampling section and each month using the *vegan* R package (Oksanen et al. 2016). Given the unequal sampling section sizes, both variables were standardized by dividing the corresponding value by the sampling section area. Spearman rank correlation coefficient (rs) between species richness and diversity was calculated.

Variation in species richness and diversity along the cave was evaluated by fitting a GAM into the data. All statistical analyses were carried out in the R environment (R Development Core Team, 2018).

Results

Environmental parameters

The annual characteristics of the environmental parameters along the cave are presented in Fig. 3. In terms of variation of climatic conditions, we distinguished three cave zones: the entrance (sampling sections 1–8), the transition (sampling sections 9–13) and the inner zone (sampling sections 14–31). The entrance zone showed the greatest variation in annual temperature (Tair, Tgr) and humidity (RH), which was considerably influenced by the external climatic conditions (Fig. 3A–C). In this illuminated zone, air temperature varied annually from –3.6 to 15.1°C, ground temperature from –0.4 to 14.4°C and relative humidity from 80.1 to 100%. The transition zone was characterized by intermediate variations of these parameters with

respect to the entrance and the inner zones, including twilight in the beginning and total darkness in the inner part of the transition zone. Air temperature varied from 6.6 to 8.5°C, ground temperature from 7.2 to 8.5°C and relative humidity was close to saturation (variation between 98.5°C and 100%). The inner, completely dark zone was relatively stable, with only limited variations in air (7.9–9.1°C) and ground temperature (8.0–9.1°C). Relative humidity remained constant at saturation. Annual mean air and ground temperature and relative humidity gradually increased, and variation in these parameters were progressively attenuated, along with increasing distance from the entrance.

The substrate moisture content (Fig. 3D) varied from 7.92 to 50.26% and carbonate content (Fig. 3E) from 0 to 53.35%. Both parameters showed no pattern with respect to distance from the entrance. The organic material varied from 2.36 to 59.79% along the cave. The greatest share (Fig. 3F) was recorded in the entrance zone, and the lowest in the middle of the cave. Disregarding the sampling section 1, with a large amount of organic matter in the floor substrate, relatively low variation of organic material among other sampling sections was found. The pH (Fig. 3G) varied from 7.82 to 8.38 and was relatively stable along the cave.

Annual and monthly species richness and diversity of troglobionts

Altogether, we recorded 1,546 individuals belonging to the following 17 troglobiont species: *Absolonia gigantea*, *Androniscus* sp., *Arrhopalites postumicus*, *Bathyscimorphus byssinus*, *Bathysciotes khevenhuelleri*, *Brachydesmus subterraneus*, cf. *Linopodes* sp., *Oncopodura cavernarum*, *Leptodirus hochenwartii*, *Neelus* sp.,

312*Neobisium spelaeum*, *Onychiuroides postumicus*, *Plusiocampa* sp., *Stalita taenaria*,
313*Titanethes albus*, *Troglopedetes pallidus*, *Zospeum spelaeum spelaeum*.

314 Cumulative standardized annual species richness and diversity of troglobionts
315showed a very high correlation (r_s=0.87, p<0.001). Both were lowest in the entrance and
316transition zones and greatest in the inner zone. However, two peaks appeared in the
317sampling section 1 in the contact zone of surface and subterranean environments, and in
318the sampling section 15 in the contact of the transition zone and the inner cave zone
319(Fig. 5A).

320 Mean standardized annual species richness and diversity of troglobionts showed
321moderate correlation (r_s=0.44, p<0.013). Comparing cumulative and standardized
322curves, they showed inverse courses. Both curves showed the lowest values at the very
323entrance, in the contact between the transition and inner zones, and at the very end of
324the cave, with two inconspicuous peaks in the entrance and inner zones (Fig. 5B). High
325correlation (r_s=0.76, p=0.004) was found between monthly species richness and
326diversity of troglobionts. There were three distinctive peaks in species richness (May–
327June 2012, August–September 2012, December 2012) and diversity (June 2012,
328September 2012, January 2013) (Fig. 6). The lowest species richness was recorded in
329July 2012 and the lowest diversity in March 2012. Both showed a trend of slight
330increasing from March 2012 until January 2013.

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332**Environmental parameters influencing the abundance of troglobionts**

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334 Data exploration revealed the presence of one outlying value in the dependent
335variable (abundance), which was removed from the dataset. The variables organic

material content, carbonate content and relative air humidity contained outliers that could not be stabilized even after the transformation. Since excluding these numerous outliers would significantly reduce the dimension of the dataset, and since data exploration revealed that these three variables were not strongly correlated with our dependent variable, we removed these three variables from the analysis. The variable passage cross-section size was log-transformed to homogenize its distribution. Air temperature, being collinear with ground substrate temperature (r>0.7), and distance from the entrance, being collinear with distance from the surface (r<0.7), were dropped from the analysis. Coplots revealed a possible interaction between distance from the entrance and the seasons. Finally, a non-linear effect of the ground temperature was detected during data exploration, and accounted for by introducing squared ground temperature as a term in the regression structure. Accordingly, we included, as variables in the initial model, the interaction between distance from the entrance and Season, log-transformed passage cross-section size, substrate moisture content, ground substrate temperature and its square, and ground substrate pH.

According to model selection based on AICc values (Table 1), the most appropriate model that explained the abundance of troglobionts had the following structure:

$$y \sim DisE*Season+Tgr+Tgr^2+MC+offset(Area)+1|Sampling\ site.$$

The combined effect of these variables on the abundance of troglobionts is illustrated in Fig. 7. The abundance of troglobionts was significantly greater near the cave entrance and decreased with increasing distance from the entrance in Spring (Season_Spring*DisE Estimated β±SE: –0.0163±0.0052, p=0.002) and Summer

(Season_Summer*DisE Estimated β±SE: –0.0183±0.0047, $p<0.001$) relative to Autumn. Instead, the abundance of troglobionts was lower and not significantly different in Winter (Season_Winter*DisE Estimated β±SE: –0.0082±0.0052, $p=0.120$). The abundance of troglobionts also increased with increasing ground substrate temperature (Tgr Estimated β±SE: 1.1850±0.3840, $p=0.002$) and decreased with squared ground substrate temperature (Tgr^2 Estimated β±SE: –0.0839±0.0279, $p=0.003$). The combination of the latter two effects resulted in a parabolic trend, with the greatest abundance of troglobionts at temperatures comprised between 7 and 10°C. Moreover, we found an increase in the abundance of troglobionts with increasing values of substrate moisture content (MC Estimated β±SE: 0.0307±0.0114, $p=0.007$).

Environmental parameters influencing the presence of troglobionts

During data exploration, no outliers were detected. The same procedure as described in detail in the previous analysis, and the same variables, except Tgr^2, were included in the initial model. According to model selection (Table 1), based on AICc values, the most appropriate model explaining the probability of the presence of troglobionts, had the following structure:

$$y \sim DisE*Season+logSize+pHgr+1|Sampling\ site.$$

The combined effect of these variables on the probability of the presence of troglobionts is illustrated in Fig. 8. The probability of the presence of troglobionts decreased significantly with increasing distance from the entrance in Spring

(Season_Spring*DisE Estimated β±SE: –0.0156±0.0057, p=0.006) and Summer (Season_Summer*DisE Estimated β±SE: –0.0146±0.0056, p=0.010) with respect to Autumn. On the other hand, the presence of troglobionts was lower in Winter, but not significantly different (Season_Winter*DisE Estimated β±SE: –0.0057±0.0055, p=0.299). We also found an increase in the probability of the presence of troglobionts with increasing log-transformed passage cross-section size (logSize Estimated β±SE: 0.5380±0.2178, p=0.013) and ground substrate pH (pHgr Estimated β±SE: 1.7096±0.8268, p=0.039).

Discussion

Subterranean habitats are suitable for studying species interactions, ecological niches, and temporal and spatial patterns in terrestrial subterranean communities. However, subterranean communities as a whole are only rarely considered in relation with environmental conditions (e.g., Di Russo et al. 1997; Pipan et al. 2011; Sendra & Reboleira 2012; Tobin et al. 2013; Bento et al. 2016; Mammola et al. 2017; Mammola & Isaia 2018). Consequently, community responses to environmental conditions are still poorly understood.

High correlations between cumulative standardized species richness and diversity of troglobionts on annual and monthly scales suggest that either of these measures provide equivalent insight into the dynamics of the troglobiotic community. In the entrance cave zone, the gradual decrease of both species richness and Shannon-Weaver diversity of troglobionts, with the highest values at the very entrance, suggests that there is an ecotone between soil and shallow karst habitats, where soil and shallow subterranean

troglobionts temporarily occur. A similar situation also occurs at the boundary between the transition and the inner cave zones. This suggests that there is another ecotone where shallow troglobionts, which regularly inhabit shallow subterranean habitats, meet deep troglobionts, regularly occurring in the inner cave zone. Thus, in the entrance cave zone, trogloxenes, troglophiles and, temporary, shallow troglobionts regularly occur, while in the inner cave zone deep troglobionts, and, temporary, troglophiles and trogloxenes occur. Culver and Pipan (2014) argued that deep soil habitats host a range of troglomorphic species, which have been largely neglected in current speleobiological studies. Until very recently, troglobionts have been discussed as a unique, convergent ecological and morphological group. However, divergence governed by competition and other factors also takes place in the subterranean habitat (Pipan & Culver 2012). The result of this study support previously published evidence (Novak et al. 2012, 2014), suggesting that there is a clear separation of troglobiotic species inhabiting shallow and deep subterranean habitats. The entrance cave zone may thus be seen as an ecotone between the surface and subterranean environments (Prous et al. 2004, 2015), the boundaries of which are poorly defined (Mammola et al. 2017) and which is characterized by relatively high fluctuation of meteorological conditions (Pipan et al. 2011). Compared to inner cave sectors, this ecotone has received only limited study (but see, e.g., Prous et al. 2004, 2015; Hobbs 2012; Culver & Pipan 2014; Yao et al. 2016; Lunghi et al. 2017). Consequently, it has remained uncovered whether deep edaphic and cryptic ground species with troglomorphic appearance should be distinguished from shallow troglobionts or not. However, further research on the life cycles, physiology and population structure of troglobionts inhabiting these two zones are required. The mean standardized species richness and diversity of troglobionts on annual scale, on the other

hand, reflect other community characteristics, referring the environmental conditions within the cave. Over the year, most favorable environmental conditions for shallow troglobionts appeared at the end of the entrance zone, while such conditions for deep troglobionts were in the middle of the inner zone. Consequently, both approaches contribute to better understanding the dynamics of troglobiont communities.

Seasonality manifests in species distributions through altering the presence and the abundance of certain species in a given place at a stated time (Murray et al. 2013; Lunghi et al. 2017). Seasonality affects many biotic functions, such as growth, feeding, and reproduction (Araújo et al. 2010; Hjernquist et al. 2012). In our study, troglobionts showed a conspicuous spatial distribution pattern that varied seasonally. In spring and summer, greater abundance and higher probability of presence were found near the entrance; these declined with increasing distance from the entrance. The same distributional pattern also applies in the case of distance from the surface, being collinear with the distance from the entrance. Although in Zguba jama, air and ground temperature and humidity, passage cross-section size and pH significantly influenced the troglobionts, this may not be the general rule in other cave types, e.g., in ice caves (cf. Raschmanová et al. 2018). A similar spatial pattern has been observed in the troglophile beetle *Sphodropsis ghilianii* (Mammola et al. 2015). Our results show that greater abundance of troglobionts occurs at ground temperatures between 7 and 10 °C and high ground substrate moisture, which may represent optimal microclimatic conditions. The optimal temperatures well fit the average annual temperature in Postojna (Postojna Meteorological Station). Such conditions were present near the entrance in spring and summer, which may in turn explain the greater abundance of troglobionts in these seasons. Our findings are in accordance with the generally

accepted understanding that troglobionts are adapted to narrow ranges of temperature and humidity (Barr & Kuehnelt 1971; Howarth 1980). They are more susceptible to desiccation, owing to their thinner exoskeleton, as compared to their surface counterparts (Howarth 1980). Furthermore, the higher availability of prey and organic matter deposits in the vicinity of the entrance (Tobin et al. 2013; Mammola & Isaia 2016) likely constitute additional essential factors determining the highest abundance of troglobionts in the entrance zone in spring and summer. In autumn and winter, lower abundance and probability of the presence of troglobionts near the entrance refer to unfavorable conditions. Most likely, these triggered the migration of troglobionts from the cave into the adjacent fissure network and partly towards climatically more stable inner cave zone. Prompt migrations, as a response to changes in microclimatic conditions, have been reported in different subterranean beetles (e.g., *Aphaenops cerberus* – Juberthie 1969; *Drimeotus* – Racoviţă 1983; *Speonomus hydrophilus* – Crouau-Roy et al. 1992; *Parapropus sericeus* and *Prospelaeobates brelihi* – Polak 2012; *Sphodropsis ghilianii* – Mammola et al. 2015). Troglobionts found near the entrance in winter are presumably adapted to more severe and variable environmental conditions. They probably possess moderate freezing tolerance, being able to withstand short exposure to sub-zero temperatures (e.g., Lencioni et al. 2010; Novak et al. 2014), a general feature of shallow (Novak et al. 2014) and some deep troglobionts (Raschmanová et al. 2018). In autumn and winter, slightly greater species richness and diversity were recorded. This might suggest that the most suitable microclimatic conditions for troglobionts occurred in these periods. On the other hand, low abundance, along with higher species richness resulted in greater diversity of troglobionts in these seasons.

Larger passage cross-section size corresponded to higher probability of the presence of troglobionts probably because these cave sections provide more microhabitat types and a calmer atmosphere. This apparently contradicts findings in some troglobiotic beetles, which show strong preference for narrow spaces but are highly sensitive to air currents in large passages (e.g., *Aphaenops* – Juberthie 1969; Juberthie & Bouillon 1983; *Speonomus* – Delay 1978; *Leptodirus*, *Anophthalmus* − Kuštor & Novak 1980a, b). In relatively small caves, such as Zguba jama, the Bernoulli principle seems to be the most important agent affecting the air current velocity leaving no calm portions in narrow passages, but allowing some calm microhabitats in more spacious cave parts.

Ground substrate pH was found to be another factor importantly affecting the presence of troglobionts; the higher the substrate pH, the greater the probability of their presence. A study on bacterial community in a cave indicated that higher substrate pH proposed a greater diversity of bacteria (Yun et al. 2016). As already reported by Vandel (1965), obligate argilivory, i.e., feeding on clay, is common in troglobiotic taxa, such as beetles of the family Leiodidae (Moldovan 2012) and the harvestman *Ischyropsalis hadzii* (own unpublished data). Leahy and Colwell (1990) found that a decrease in pH from 8.5 to 7.4 significantly increased the decomposition rates of organic matter by microbes, which might consequently result in lower food availability for troglobionts. Organic matter did not affect the distribution of troglobionts, since, except the humus accumulation at the entrance, there were no further accumulations of organic matter, and its contents in the floor substrate was relatively low and stable along the cave.

It is worth noting that abundance and richness data deriving from baited pitfall-trapping must be carefully considered since these results might differ in comparison to other methods (Poulson & Culver, 1968; Juberthie, 1969; Kuštor & Novak, 1980a, b;

Weinstein & Slaney, 1995; Culver & Pipan, 2009; Kozel et al. 2017; Wynne et al., 2019). However, in previous studies (Novak et al. 2012; Kozel et al. 2017) we showed that a monthly two-day setting of the traps being 3–4 m distant from each other provides enough data for many statistical analyses, along with any detectable influence on the depletion of the fauna.

Our finding that most troglobiotic species preferred the inner, climatically the most stable, cave zone, is in accordance with general trends that species richness and diversity of troglobionts are typically greatest deeper inside caves (Schiner 1854; Racoviţă 1907; Poulson & White 1969; Howarth 1983; Tobin et al. 2013). However, this is in contrast to Novak et al. (2012), who found that most troglobionts are distributed within the upper 10 m of subsurface strata. This difference in the findings might be the consequence of either comparing only one (our study) vs. a large number of caves (Novak et al. 2012) or due to different sampling efforts (monthly vs. seasonal sampling).

In conclusion, in speleological terms, Zguba jama is a relatively short cave, shallow beneath the surface. The mean annual air temperature roughly corresponds to the mean annual air temperature outside. The other environmental characteristics, such as relative humidity, substrate moisture content, organic matter content and pH, are typical of caves in the region (own unpublished data). Although partitioning caves into the entrance, intermediate and inner zones is conceptually clear, in caves some practical guidelines are needed. In moderate climates, the entrance cave zone is illuminated, the air and ground temperatures may fall below 0°C and the relative humidity is nearly all the time below 100 %. The intermediate zone is a twilight or completely dark zone, where the temperatures never fall below 0°C, while the relative humidity may be lower than 100

% at least in one season. The inner zone is completely dark and thermally (temperature variation usually less than 1°C; Cigna 2002) and humidity stable (saturated or subsaturated). In most caves the three zones can only be identified after checking the illumination, and measuring annual variation of air and ground temperature and relative humidity at least once each season. Two ecotones: the entrance and the inner one can be distinguished in the cave, the first one sharing the edaphic and shallow troglobiont faunas, and the second one the shallow troglobiont and deep troglobiont faunas. Cumulative and standardized data on species richness and diversity reveal different population properties; consequently, both approaches should be applied in future studies to get a better insight in the dynamics of subterranean fauna. In this study we identified the most important environmental parameters affecting spatio-temporal dynamics of troglobionts in the cave. Hosting a relatively rich fauna (Kozel et al. 2017) and being easily modeled due to its horizontal development and simple shape, this cave well serves as an amenable model to study the annual distributional dynamics of troglobionts in shallow caves.

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790**Fig. 1.** Geographical location of the study cave (source: LIDAR DEM, Cave Register
7912018, TTN5).
792

793**Fig. 2.** The ground plan of Zguba jama showing sampling sections (red lines) and
794pitfall-trap sites (blue squares) with a pair of the ground and the upper trap. Modified
795from Šebela (1994) and Kozel et al. (2017).
796

797**Fig. 3. (A–D)** Annual mean and range of monthly measured environmental parameters;
798**(E–G)** parameters measured only once along the cave.
799

800**Fig. 4. (A)** Cumulative standardized annual species richness and the Shannon-Weaver
801index (H') of troglobionts per sampling section. Regression lines were obtained by
802fitting a GAM into the data (annual species richness: df=3, F=2.91, p=0.053; Shannon-
803Weaver index: df=3, F=5.73, p=0.004). **(B)** Mean±SD standardized annual species
804richness and Shannon-Weaver index (H') of troglobionts per sampling section.
805Regression lines were obtained by fitting a GAM into the data (annual species richness:
806df=3, F=8.39, p<0.001; Shannon-Weaver index: df=3, F=2.63, p=0.071). For better
807visualization, confidence intervals are not shown.
808

809**Fig. 5.** Monthly species richness and values of the Shannon-Weaver index (H′) of
810troglobionts in Zguba jama.

Fig. 6. Predicted values of the effects of distance from the entrance in interaction with the Season and **(A)** ground substrate temperature, and **(B)** substrate moisture content on the abundance of troglobionts obtained from the Generalized Linear Mixed Model (GLMM). In each panel, to generate the prediction, the unplotted variable is set at its mean value.

Fig. 7. Predicted values of the effects of distance from the entrance in interaction with the Season and **(A)** log-transformed passage cross-section size, and **(B)** ground substrate pH on the probability of the presence of troglobionts obtained from the Generalized Linear Mixed Model (GLMM). In each panel, to generate the prediction, the unplotted variable is set at its mean value.

Table 1. Model selection according to the corrected Akaike Information Criterion (AICc) and the Aikake weight. Models are ordered from the most to the least appropriate. Df, degrees of freedom; w_i (AICc), Akaike weight.

Model structure	Df	AICc	w_i (AICc)
Troglobionts abundance models			
y~DisE*Season+Tgr+Tgr^2+MC+offset(Area)+1|Sampling section	13	1633.11	0.64
y~DisE*Season+Tgr+Tgr^2+MC+logSize+offset(Area)+1|Sampling section	14	1635.20	0.22
y~DisE*Season+Tgr+Tgr^2+MC+pH+logSize+offset(Area)+1|Sampling section	15	1636.17	0.14
Troglobionts presence-absence models			
y~DisE*Season+logSize+pH+1|Sampling section	11	420.41	0.56
y~DisE*Season+logSize+pH+MC+1|Sampling section	12	421.72	0.27
y~DisE*Season+logSize+pH+MC+Tgr+1|Sampling section	13	422.47	0.13